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ORIGINAL RESEARCH

Systemic inflammation and liver damage in HIV/hepatitis C virus coinfection

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Objectives

Chronic hepatitis C virus (HCV) and HIV viral infections are characterized by systemic inflammation. Yet the relative levels, drivers and correlates of inflammation in these settings are not well defined.

Methods

Seventy-nine HIV-infected patients who had been receiving antiretroviral therapy (ART) for more than 2 years and who had suppressed plasma HIV levels (< 50 HIV-1 RNA copies/mL) were included in the study. Two patient groups, HCV-positive/HIV-positive and HCV-negative/HIV-positive, and a control group comprised of healthy volunteers (n = 20) were examined. Markers of systemic inflammation [interleukin (IL)-6, interferon gamma-induced protein (IP)-10, soluble tumour necrosis factor receptor-I (sTNF-RI) and sTNF-RII], monocyte/macrophage activation [soluble CD163 (sCD163), soluble CD14 and neopterin], intestinal epithelial barrier loss [intestinal fatty acid binding protein (I-FABP) and lipopolysaccharide (LPS)] and coagulation (D-dimers) were analysed. CD4 naïve T cells and CD4 recent thymic emigrants (RTEs) were enumerated.

Results

Plasma levels of IP-10, neopterin and sCD163 were higher in HCV/HIV coinfection than in HIV monoinfection and were positively correlated with indices of hepatic damage [aspartate aminotransferase (AST), alanine aminotransferase (ALT) and the AST to platelet ratio index (APRI)]. Levels of I-FABP were comparably increased in HIV monoinfection and HIV/HCV coinfection but LPS concentrations were highest in HCV/HIV coinfection, suggesting impaired hepatic clearance of LPS. Plasma HCV levels were not related to any inflammatory indices except sCD163. In coinfected subjects, a previously recognized relationship of CD4 naïve T-cell and RTE counts to hepatocellular injury was defined more mechanistically by an inverse relationship to sCD163.

Conclusions

Hepatocellular injury in HCV/HIV coinfection is linked to elevated levels of certain inflammatory cytokines and an apparent failure to clear systemically translocated microbial products. A related decrease in CD4 naïve T cells and RTEs also merits further exploration.

Keywords: antigens, CD31, hepatitis C, highly active antiretroviral therapy, HIV infections, inflammation mediators

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Introduction

An estimated 10–15% of the 35 million people living with HIV infection world-wide are also infected with hepatitis C virus (HCV) [1]. These two viral diseases

can adversely influence each other. HIV speeds up the course of HCV infection, accelerating liver fibrosis and cirrhosis, and promoting liver cancer [2,3]. In turn, HCV coinfection has been linked to CD4 and CD8 T-cell activation [4,5] and increased CD4 T-cell apoptosis [6,7], and in some studies has been associated with diminished CD4 T-lymphocyte restoration with antiretroviral therapy (ART) [8].

Indices of systemic inflammation and coagulation are now recognized as important predictors of morbidity and mortality in treated HIV infection [9–11]. Here, we investigated whether HIV-infected patients with suppressed viraemia on combination ART have different systemic levels of inflammation or coagulation from HCV-coinfected patients and, if so, whether these levels are related to indices of hepatic damage.

Patients and methods

This work was approved by the Institutional Review Board of Perm Regional Center for Protection against AIDS and Infectious Diseases (IRB00008964). All patients provided their written informed consent.

Seventy-nine HIV-infected patients receiving ART for more than 2 years and 20 healthy controls participated in the study. All patients had a confirmed diagnosis of HIV infection, were adherent to their ART regimen, and had plasma HIV RNA levels < 50 HIV-1 RNA copies/mL. ART regimens included two nucleoside reverse transcriptase inhibitors (NRTIs) together with a ritonavir-boosted protease inhibitor or a nonnucleoside reverse transcriptase inhibitor. HCV coinfection was confirmed by the demonstration of HCV RNA in plasma using a polymerase chain reaction (PCR)-based assay (Quantitative RT-Gepatogen C kit; DNA Technology, Moscow, Russia); HCV-uninfected subjects each had a negative test for serum antibodies to HCV. Patients who had been exposed to interferon/ribavirin treatment were excluded from the study. HIV infection duration was timed from the date of the first positive western blot analysis. HCV infection duration was calculated from when the first positive enzyme-linked immunosorbent assay (ELISA) result was received. A report describing the lymphocyte phenotype in these subjects has been published previously [12].

Three groups were included in the study: (1) HIV/HCV-coinfected patients (n = 42); (2) HIV-monoinfected patients (n = 37); (3) uninfected volunteers (n = 20). There were no differences between the two infected groups in nadir CD4 T-cell count (Table 1) or prior AIDS-defining conditions. No information on alcohol consumption and smoking was provided.

HIV and HCV levels in plasma

Plasma levels of HIV RNA were assessed using a Versant 440 amplifier (Siemens, Washington, D.C, USA) and Versant HIV 1 RNA 3.0 assay b kits (Bayer, Leverkusen, Germany). HCV RNA levels in plasma were measured using an iCycler IQ5 (Bio-Rad, Hercules, CA, USA) and real-time PCR Quantitative RT-Gepatogen C kits (DNA Technology).

Blood samples for T-cell phenotyping

Approximately 30 mL of blood was collected from each participant in Vacutainer tubes containing Ethylenediaminetetraacetic acid (EDTA) (Becton Dickinson, Franklin Lakes, NJ, USA). CD4 T-cell numbers were counted in real time using the IMK-Lymphocyte Kit (Becton, Dickinson and Company, San Jose, CA, USA) and a BD FACSCalibur flow cytometer. (Franklin Lakes, NJ, USA) Peripheral blood mononuclear cells (PBMCs) were isolated using Diacoll-1077 (Dia-M, Moscow, Russia) density sedimentation. PBMCs were cryopreserved in fetal calf serum and dimethyl sulfoxide, and then stored at −196°C.

Monoclonal antibodies

Fluorochrome-tagged monoclonal antibodies (anti-CD3-PerCP, anti-CD4-AF700, anti-CD27-APC-Cy7, anti-CD45RA-APC, anti-CCR7-PE-Cy7 and anti-CD31-FITC) and isotype control antibodies were obtained from Becton Dickinson. A Live/Dead Fixable Yellow Dead Cell Stain Kit was obtained from Life Technologies (Grand Island, NY, USA).

Flow cytometry

Peripheral blood mononuclear cells (PBMCs) were thawed and stained and viable cells were enumerated using a Becton Dickinson LSR II Flow Cytometer. Naïve CD4 T cells were identified as $CD3^+CD4^+CD27^+CD45RA^+CCR7^+$. Naïve $CD31^+$ T lymphocytes were considered to be recent thymic emigrants (RTEs). At least 100 000 events in the lymphocyte gate were collected for each sample. Relative values were determined from the cytometer data. Absolute lymphocyte subpopulations were calculated based on CD4 T-cell numbers detected in fresh blood.

ELISA

ELISA kits for the detection of interleukin-6 (IL-6), interferon gamma-induced protein-10 (IP-10), soluble CD163 (sCD163), soluble CD14 (sCD14), soluble tumour necrosis

Table 1 Clinical characteristics of HIV/hepatitis C virus (HCV)-coinfected and HIV-monoinfected patients

Characteristic	HIV/HCV coinfected	HIV monoinfected	Uninfected
Examined subjects (*n*)	42	37	20
Age (years) [median (IQR)]*	33 (32–37)	34 (31–41)	31 (26–35)
Male gender [*n* (%)]	26 (61.9)	8 (21.6)	8 (40.0)
HIV transmission route [*n* (%)]			
Injecting drug use	36 (85.7)	1 (2.7)	–
Sexual	6 (14.3)	36 (97.3)	–
Homosexuals	0	0	0
Sex workers	0	0	0
Active drug users	0	0	0
HIV infection characteristics			
Infection duration (years) [median (IQR)]	11 (9–12)	8 (6–10)	–
	$P_{1-2} < 0.001$		
HAART duration (years) [median (IQR)]	3.5 (2–5)	4 (3–5)	–
	$P_{1-2} > 0.05$		
Nadir CD4 count (cells/μL) [median (IQR)]	140 (100–170)	150 (106–170)	–
	$P_{1-2} > 0.05$		
CD4 count at study entry (cells/μL) [median (IQR)]	350 (260–450)	410 (290–570)	1050 (660–1280)
	$P_{1-2} > 0.05$	$P_{2-3} < 0.001$	$P_{1-3} < 0.001$
HIV viral load (copies/mL) [median (IQR)]	< 50	< 50	–
HCV infection characteristics			
Infection duration (years) [median (IQR)]	11 (8–12)	–	–
HCV viral load (log_{10} copies/mL) [median (IQR)]	6.21 (2.88–6.59)	< 2.88	< 2.88
AST (U/L) [median (IQR)]	47 (29–75)	19 (17–23)	19 (15–24)
	$P_{1-2} < 0.001$	$P_{2-3} > 0.05$	$P_{1-3} < 0.001$
ALT (U/L) [median (IQR)]	59 (28–112)	18 (14–23)	19 (15–26)
	$P_{1-2} < 0.001$	$P_{2-3} > 0.05$	$P_{1-3} < 0.001$
γ-GT (U/L) [median (IQR)]	71 (35–122)	30 (23–45)	27 (21–34)
	$P_{1-2} < 0.001$	$P_{2-3} > 0.05$	$P_{1-3} < 0.001$
Albumin (g/L) [median (IQR)]	41.7 (40.9–42.5)	41.3 (40.4–43.5)	41.8 (40.8–42.6)
	$P_{1-2} > 0.05$	$P_{2-3} > 0.05$	$P_{1-3} > 0.05$
Platelets (10^9/L) [median (IQR)]	202 (167–244)	234 (177–276)	–
	$P_{1-2} > 0.05$		
APRI [median (IQR)]	0.6 (0.4–1.2)	0.2 (0.2–0.3)	–
	$P_{1-2} < 0.001$		

AST, aspartate aminotransferase; ALT, alanine aminotransferase; APRI, AST-to-platelet ratio index; HAART, highly active antiretroviral therapy; IQR, interquartile range; γ-GT, γ-glutamyl transpeptidase.
*Median with interquartile range (25th–75th percentile).
Statistical analyses were carried out using the Mann–Whitney test.

factor receptor-I (sTNF-RI), soluble tumour necrosis factor receptor-II (sTNF-RII) and intestinal fatty acid binding protein (I-FABP) were purchased from R&D Systems (Minneapolis, MN, USA). D-dimer kits were purchased from Diagnostica Stago (Asnieres, France). Neopterin competitive ELISA kits were purchased from IBL International (Hamburg, Germany). Lipopolysaccharide (LPS) levels were assessed using a Hycult Biotech Limulus amebocyte lysate chromogenic endpoint assay kit (Uden, the Netherlands). Assays were performed according to the kit instructions. Plasma samples were diluted as needed to assure that results were within the linear range of the assay.

Statistical analysis

Data are reported as medians and interquartile ranges. Groups were compared using the Mann–Whitney test. Multiple regression analysis was used to control for the effects of possible confounding factors. Correlation analysis was performed using the Spearman method. All statistical analyses were performed using STATISTICA 6.0 software (Dell Software, Round Rock, TX, USA).

Results

Clinical characteristics

The ages of the HIV-infected patient groups and healthy controls were comparable (Table 1). The median age was 33 years in the HIV/HCV-coinfected group, 34 years in the HIV-monoinfected group and 31 years in the uninfected group. Men were overrepresented (61.9%) among HIV/HCV-coinfected patients, reflective of the features of the HCV infection epidemic in Russia [13]. In contrast, the HIV-monoinfected patients in this study were predominantly (78.4%) women. The difference in the gender ratio was significant ($P < 0.001$). In the healthy control group, 40% were

men. The known duration of infection was longer in coinfected subjects than in HIV-monoinfected subjects (11 *vs.* 8 years, respectively). There were no differences between the two infected groups in CD4 T-cell numbers before or after ART initiation. In HIV/HCV-coinfected subjects, the median HCV RNA levels exceeded 1 000 000 copies/mL and liver enzymes were elevated compared with the levels in HIV-monoinfected patients, while albumin levels and platelet counts in coinfected and monoinfected persons were not significantly different. The AST to platelet ratio index (APRI) for predicting fibrosis and cirrhosis [14] was higher in the HIV/HCV-coinfected group than in the HIV-monoinfected group.

Systemic inflammation indices are elevated in HCV/HIV coinfection

Plasma levels of the inflammatory cytokines IL-6 and IP-10, the monocyte/macrophage markers neopterin and sCD163, and sTNF-RII were higher in HIV/HCV-coinfected patients than in patients who were HIV-monoinfected and, except for sTNF-RII, were also higher than in healthy controls (Fig. 1). As the two infected groups differed in the duration of HIV infection and gender composition, we investigated whether these factors might have confounded our results. After adjustment for these factors, the difference between the two HIV-positive groups in the levels of IL-6 and sTNF-RII lost statistical significance. With correction for gender and duration of HIV infection, levels of IP-10, sCD163 and neopterin remained significantly higher in HCV/HIV-coinfected subjects than in HIV monoinfection. Median levels of IP-10, sCD163 and sTNF-RII in HIV-monoinfected subjects were not different from those in healthy controls, but IL-6, neopterin and sCD14 levels were higher in HIV-monoinfected patients than in healthy subjects. These differences in inflammatory markers may be associated with intestinal epithelium damage, as plasma I-FABP levels in both groups

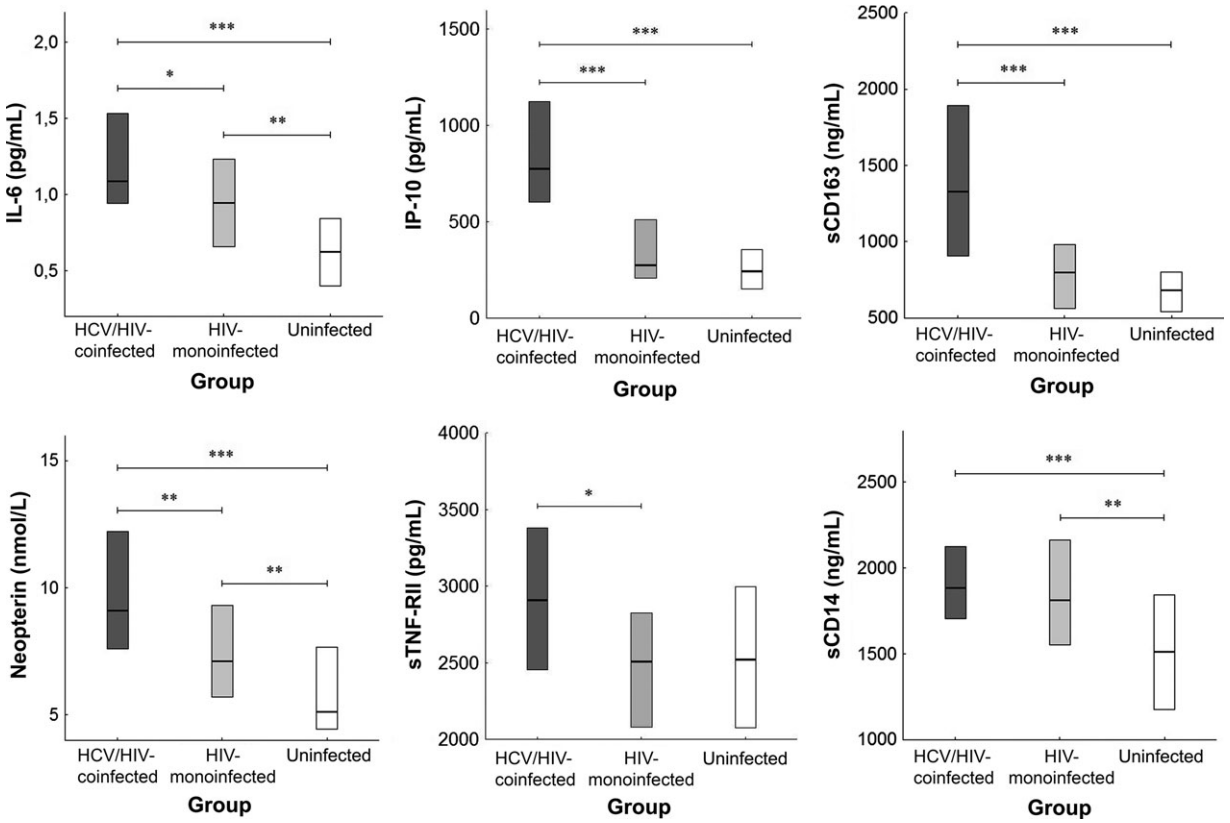

Fig. 1 Plasma indicators of systemic inflammation in hepatitis C virus (HCV)/HIV-coinfected patients. Plasma concentrations of interleukin (IL)-6, interferon gamma-induced protein (IP)-10, soluble CD163 (sCD163), neopterin, soluble tumour necrosis factor receptor-II (sTNF-RII) and soluble CD14 (sCD14) are shown in three patient groups: patients coinfected with HCV/HIV, HIV-monoinfected patients and healthy volunteers without HIV or HCV infection. Columns with horizontal lines show medians with interquartile ranges. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$ (Mann–Whitney test).

of HIV-infected patients were also significantly higher ($P < 0.01$) than those in the control group, and plasma LPS concentrations were higher in HCV/HIV-coinfected subjects than in HIV-monoinfected patients and uninfected controls (Fig. 2). In contrast, plasma D-dimer levels, reflecting coagulation and fibrinolysis, were similar among the three groups.

To explore the possibility that HCV coinfection increases monocyte/macrophage activation (sCD163 and neopterin levels) and stimulates interferon (IFN)-dependent production of the chemokine IP-10, we assessed the relationship of these markers to indices of HCV replication and hepatic injury.

In the group of HCV/HIV-coinfected patients, we found highly significant and consistent correlations between indices of hepatic damage [AST, ALT and APRI] and plasma IP-10, sCD163 and neopterin (Fig. 3). Correlations with levels of sCD14, I-FABP, sTNF-RI and sTNF-RII were not significant. AST levels correlated with levels of D-dimers, albeit weakly ($R = 0.326$; $P < 0.05$). HCV levels in plasma were also associated with liver enzyme elevations [$R_{AST–HCV} = 0.527$ ($P < 0.001$), $R_{ALT–HCV} = 0.483$ ($P < 0.01$) and $R_{APRI–HCV} = 0.361$ ($P < 0.05$)], but, among all the markers of systemic inflammation, correlated only with sCD163 concentration ($R_{sCD163–HCV} = 0.316$; $P < 0.05$).

In an earlier report in this cohort, we found inverse significant relationships between the magnitude of hepatic damage (ALT, AST and APRI) and absolute numbers of circulating CD4 RTEs [12]. Having found that indices of hepatocellular injury are linked to inflammatory markers in HIV/HCV coinfection, we examined here the relationship between these inflammatory markers and CD4 RTEs, and found that higher levels of sCD163 were associated with fewer circulating CD4 RTEs and with fewer CD4 naïve T cells (Fig. 4).

Discussion

HIV and HCV infections are each characterized by increases of various inflammatory marker levels in the blood [15–17]. With suppressive ART, plasma concentrations of inflammatory markers tend to decrease but do not always normalize [18]. Here, we compared plasma levels of inflammatory and coagulation markers in HIV-infected and HIV/HCV-coinfected patients who were receiving suppressive ART. In both groups, HIV levels in plasma were suppressed while HCV replication was uncontrolled, providing an opportunity to explore the effects of HCV replication in the setting of chronic HIV infection while attenuating the direct effects of HIV replication. Plasma concentrations of IL-6, IP-10, sCD163, neopterin and sTNF-RII were significantly higher in coinfected subjects than in HIV-infected patients not infected with HCV. As the patient groups were not comparable in gender composition and known duration of HIV infection, adjustment for these two factors left only differences in IP-10, sCD163 and neopterin remaining significantly and independently higher in HCV/HIV-coinfected patients than among HIV-infected subjects not infected with HCV. Although a contribution of ART-induced hepatotoxicity in the setting of HCV/HIV coinfection cannot be excluded, a simpler and more plausible explanation is that the observed effects are related to HCV/HIV-mediated liver damage [19,20].

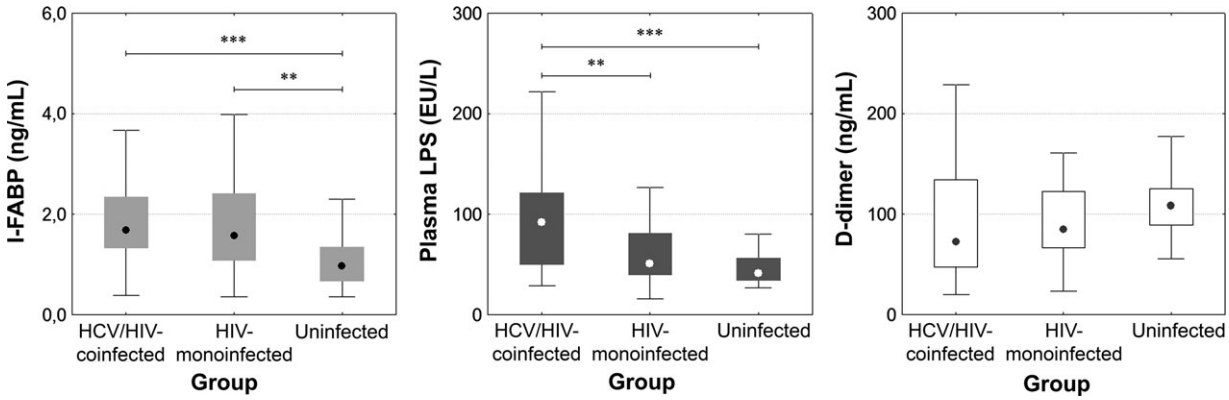

Fig. 2 Plasma concentrations of intestinal fatty acid binding protein (I-FABP), lipopolysaccharide (LPS) and D-dimers in hepatitis C virus (HCV)/HIV-coinfected and HIV-monoinfected patients. Three patient groups are shown: HCV/HIV-coinfected patients, HIV-monoinfected patients and healthy uninfected volunteers. Medians, interquartile ranges, and upper and lower ranges are presented. $^{**}P < 0.01$; $^{***}P < 0.001$ (Mann–Whitney test).

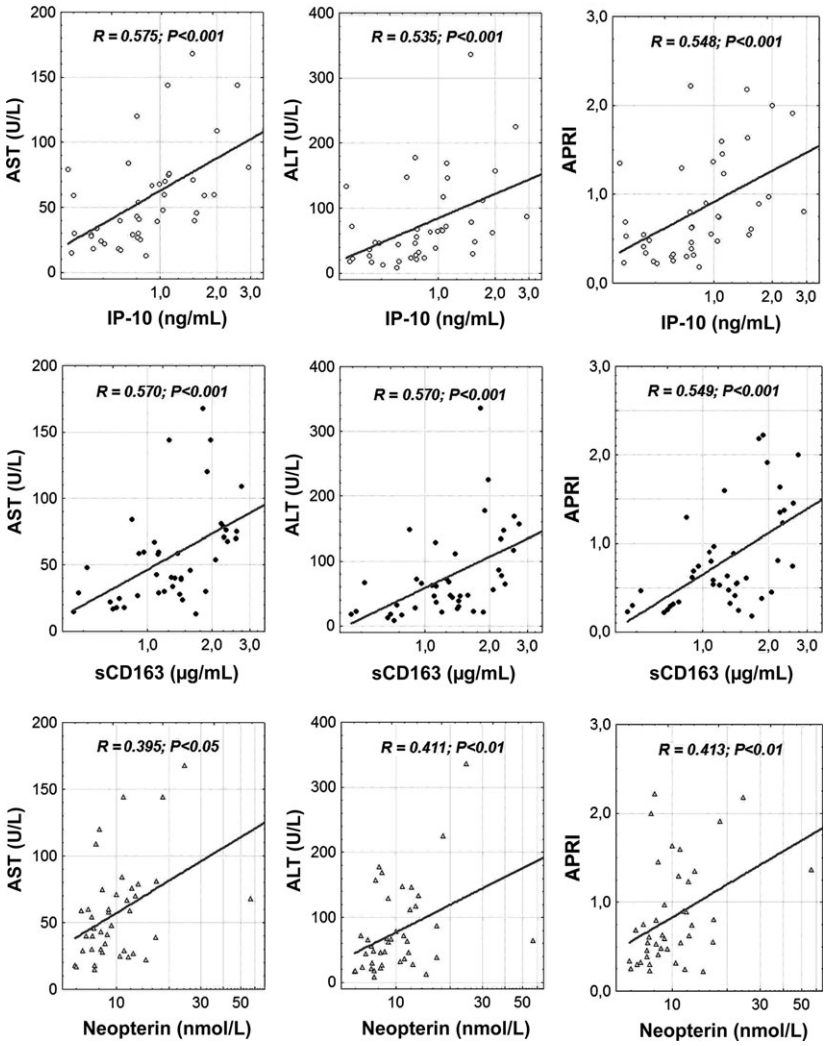

Fig. 3 Relationship between hepatocellular damage and indices of systemic inflammation. The correlation analysis was performed using the Spearman method. ALT, alanine aminotransferase; AST, aspartate aminotransferase; APRI, AST to platelet ratio index; IP-10, interferon gamma-induced protein-10.

Whereas there are numerous sources of IP-10, in HCV infection, IP-10 is synthesized by liver sinusoidal lining cells [21] and is induced by interferons and co-stimulated by TNF-α and IL-1 [22,23]. The primary role of the chemokine IP-10 is to recruit CD4 Type 1 helper T cells (Th1), CD8 cytotoxic T cells, and natural killer (NK) cells through interaction with Chemokine (C-X-C motif) receptor 3 (CXCR3) to provide a proinflammatory antiviral immune response [24–27].

During chronic HCV infection, an increase in the number of macrophage-like Kupffer cells is observed [28,29]. These cells acquire an activation phenotype [30], and express greater levels of CD33 and CD163 [31,32]. Blood levels of sCD163 may reflect systemic macrophage activation. Higher levels of sCD163 are seen in subjects with HCV/HIV coinfection than in HIV-monoinfected patients [33] and in HCV infection are linked to the development of cirrhosis [34].

While effective ART does not reliably result in complete suppression of immune activation and inflammatory responses in HIV-infected patients [35,36], we found here that plasma levels of IL-6, neopterin and sCD14 in subjects not coinfected with HCV remained higher than in controls, while levels of IP-10, sCD163 and TNF-RII were similar to control levels. The drivers of persistent inflammation in treated HIV infection and HIV/HCV coinfection are not entirely clear but, in these settings, damage to the gut epithelial barrier has been implicated in promoting

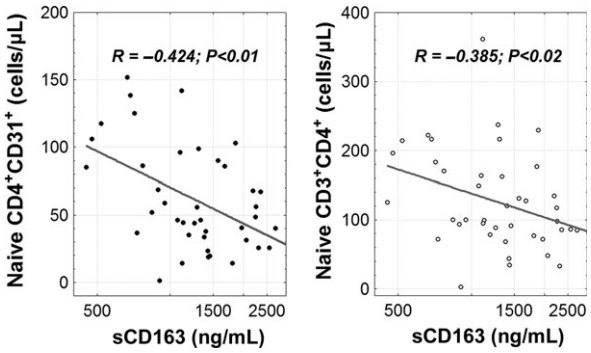

Fig. 4 Relationship between numbers of CD4 recent thymic emigrants, naïve CD4 T-cell counts and plasma sCD163 levels in hepatitis C virus (HCV)/HIV-coinfected subjects. The correlation analysis was performed using the Spearman method.

translocation of microbial products from the gut lumen into the systemic circulation [37]. Elevated plasma levels of I-FABP are regarded as a marker of this intestinal damage [19,38]. Interestingly, we found increased plasma levels of LPS in HIV infection and even higher levels in HIV/HCV coinfection, while in HIV monoinfection and HIV/HCV coinfection the elevated levels of I-FABP and the LPS co-receptor sCD14 were comparable. These data suggest that in HIV-infected/HCV-uninfected and HIV/HCV-coinfected individuals the gut barrier defect may be comparable, allowing bacterial products access to the portal vein. However, impaired hepatic clearance of LPS in HCV coinfection may result in even higher levels of LPS in the peripheral blood of HIV/HCV-coinfected patients compared with HIV-infected patients not infected with HCV. It is also possible that the impaired clearance of LPS and other microbial products not measured here may contribute to the marked increases in other inflammatory mediators that we found in HCV/HIV coinfection compared with levels in HIV-infected patients negative for HCV.

While sCD14 levels were elevated in both HIV infection and HCV/HIV coinfection, there was no significant difference in sCD14 levels between HIV monoinfection and HIV/HCV coinfection. In contrast, Sandler *et al.* [19] found that sCD14 level was correlated with markers of hepatic destruction (AST) and abnormal liver function (γ-glutamyl transpeptidase, alkaline phosphatase and α-fetoprotein), and French *et al.* [39] showed that levels of sCD14 were higher in HIV/HCV-coinfected women during periods of liver disease progression than during intervals when minimal or no progression occurred. We did not find a relationship between sCD14 and any indices of hepatocellular damage in this cohort, where APRI scores are consistent with a low probability of advanced liver damage. However, we found here that plasma levels of two other markers of macrophage activation, sCD163 and neopterin, and levels of the IFN-inducible protein IP-10 were significantly higher in HCV/HIV coinfection than in HIV-infected patients not infected with HCV and, while only sCD163 levels were correlated with plasma levels of HCV, each was correlated with three indices of hepatic damage (AST, ALT and APRI).

In earlier works, we and others found an inverse relationship between indices of hepatocellular damage and the frequency of circulating CD4 T-cell RTEs as determined by the expression of CD31 [12,40]. In the current work, we found that circulating levels of sCD163, which are linked to indices of hepatocellular inflammation, were also correlated inversely with the numbers of circulating naïve CD4 T cells and CD4 RTEs. The relationship among these indices remains incompletely understood but it is possible that processes taking place in the liver may play a role in alteration of CD4 T-cell recovery during ART in the setting of HCV/HIV coinfection.

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Conflicts of interest: The authors have no conflicts of interest to disclose.

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